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Effects of Low-Intensity Microwave Radiation on Oxidant-Antioxidant Parameters and DNA Damage in the Liver of Rats

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The continuously increasing usage of cell phones has raised concerns about the adverse effects of microwave radiation (MWR) emitted by cell phones on health. Several in vitro and in vivo studies have claimed that MWR may cause various kinds of damage in tissues. The aim of this study is to examine the possible effects of exposure to low-intensity MWR on DNA and oxidative damage in the livers of rats. Eighteen Sprague–Dawley male rats were divided into three equal groups randomly ($n = 6$). Group 1 (Sham-control): rats were kept under conditions the same as those of other groups, except for MWR exposure. Group 2: rats exposed to 1800 MHz (SAR: 0.62 W/kg) at 0.127 ± 0.04 mW/cm^2 power density, and Group 3: rats exposed to 2,100 MHz (SAR: 0.2 W/kg) at 0.038 ± 0.03 mW/cm^2 power density. Microwave application groups were exposed to MWR 2 h/day for 7 months. At the end of the exposure period, the rats were sacrificed and DNA damage, malondialdehyde (MDA), 8-hydroxydeoxyguanosine (8-OHdG), and total oxidant-antioxidant parameter analyses were conducted in their liver tissue samples. It was found that 1800 and 2100 MHz low-intensity MWR caused a significant increase in MDA, 8-OHdG, total oxidant status, oxidative stress index, and comet assay tail intensity ($P < 0.05$), while total antioxidant status levels ($P < 0.05$) decreased. The results of our study showed that whole-body exposure to 1800 and 2100 MHz low-intensity MWR emitted by cell phones can induce oxidative stress by altering oxidant-antioxidant parameters and lead to DNA strand breaks and oxidative DNA damage in the liver of rats. Bioelectromagnetics. 2021;42:76–85. © 2020 Bioelectromagnetics Society

Keywords: **microwave radiation; liver; DNA damage; 8-OHdG; MDA**

INTRODUCTION

Cell phones, which are rapidly increasing day by day in terms of the number of users and duration of use, emit microwave radiation (MWR) in the form of non-ionization electromagnetic radiation. As a result, concerns have increased about the possible dangers of electromagnetic field radiation radiated by cell phones affecting human health [Dasdag and Akdag, 2016; Akdag et al., 2018; Yang et al., 2020; Smith-Roe et al., 2020]. Today, intense research has been conducted on many biological effects of electromagnetic waves. It is known that biological systems give different biological responses to electromagnetic wave applications at different frequencies and intensities [Topal et al., 2015]. Cellphones emit low-intensity MWR at frequencies between 800 and 2,200 MHz, which can be absorbed by different body organs depending on where they are carried on the body [Bhargava et al., 2012]. In our previous studies, we determined that both 1800 and 2100 MHz frequencies had extremely harmful effects on brain and testicle tissues [Alkis et al., 2019a,b]. Therefore, we utilized these frequencies in this study for the liver. The liver is very sensitive to the impact of reactive oxygen species (ROS), and MWR mainly undergoes detoxification within the human liver [Celik et al., 2016]. In addition, recently it has been preferred to use wireless Bluetooth or corded headsets instead of

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connecting cell phones directly to the ear. However, when people use a cell phone in this way, they usually carry the phone in the front pocket of the pants, in the hand, or on the belt [Alkis et al., 2019b]. Thus, the liver remains under the influence of cell phone radiation in speech mode for a long time. When the cell phone is carried on the belt or in the front pocket, its antenna's proximity to the liver and other abdominal organs has increased concerns about the biological interactions between the MWR and internal organs [Meo et al., 2010; Eid et al., 2015].

The formation/breaking processes of hydrogen bonds to electromagnetic fields (EMFs) occurring in cell aqua medium [Chaplin, 2006; Ayrapetyan, 2017] is highly sensitive, especially in the metabolism-driven collective dynamics of intra-cellular water molecules [Ayrapetyan and De, 2014]; and this sensitivity renders the function of the individual biochemical reactions nonessential in EMF's non-thermal (NT) effect on intra-cellular metabolism. In this regard, the water hypothesis appears to be more reliable to explain EMF's biological effect. The valence angle of water molecules between O–H bonds, which is the determining factor for its physicochemical characteristics and dissociation, displays high sensitivity to various environmental factors [Chaplin, 2006; Ayrapetyan, 2017]; according to this assumption, EMF can modulate the cell metabolism by means of altering the physicochemical characteristics of water, a common medium for the metabolic reactions in organisms and cells. In their studies, Ayrapetyan [2015] revealed that the EMF's effect on the physicochemical characteristics of water solutions and the formation of ROS depends on the gas composition of the medium, light, temperature, Ca^{2+} concentration in the water solution, and background radiation. The formation of H_2O_2 in physiological solution (PS) upon the impact of EMF is one of the major harbingers of the modulation of cell metabolism [Ayrapetyan et al., 2005; Gapeyev et al., 2009].

Several studies have indicated that MWR may lead to an undesired impact in liver tissue such as DNA strand breaks [Trosic et al., 2011], lipid peroxidation [Celik et al., 2016], oxidative stress [Ozgur et al., 2010], intense pro-oxidant process, and decreased antioxidant enzyme activity [Sieroń-Stołtny et al., 2017]. The harmful effects of microwaves on several tissues exposed to the EMF demonstrated in empirical studies are usually caused by increasing body temperature [Esmekaya et al., 2011], free radical concentration, and deterioration of oxidant/antioxidant balance [Yakymenko et al., 2015; Chauhan et al., 2017]. The balance between free radicals and antioxidants deteriorates in favor of free radicals and oxidative damage known as oxidative stress, which occurs because of excessive production of free radicals and insufficiency of antioxidants [Halliwell and Gutteridge, 2015]. Oxidative stress may cause severe metabolic dysfunctions such as peroxidation of membrane lipids, depletion of nicotine amide nucleotides, the rise of intra-cellular Ca^{2+} ions, cytoskeleton degradation, and DNA damage [Guler et al., 2010]. As the repair of almost all biomolecules depends on the information encoded in DNA, oxidative DNA damage has special importance. 8-hydroxydeoxyguanosine (8-OHdG) is the most commonly used base adduct formed by free radical attacks [Yokus et al., 2005]. Another metabolic dysfunction of oxidative stress is the peroxidation of lipids. One of the main biological markers commonly used in the determination of oxidative degradation in lipids is malondialdehyde (MDA) [Tsikas, 2017]. Despite several studies, it is still unclear whether low-intensity MWR emitted by cell phones is causing serious problems in the liver. The goal of this study is to investigate the impact of long-term exposure of low-intensity MWR on oxidant-antioxidant parameters, DNA damage, and lipid peroxidation in rat's liver.

MATERIALS AND METHODS

Animals and Microwave Radiation Exposure

A total of 18 4-month-old Sprague–Dawley male rats were taken from the Health Sciences Research and Application Center at Dicle University. The rats were assigned to three equal groups randomly (*n*: 6). Group 1 was appointed as a sham-control group, Group 2 was appointed as an application group exposed to 1800 MHz, and Group 3 was appointed as an application group exposed to 2,100 MHz microwave. The animals were hosted in a laboratory medium at 12/12 h dark/light period, at 22 ± 2 °C and 45%–50% relative humidity. The rats were fed *ad libitum* with standard tap water and pellets (Tavas, Adana, Turkey). The experimental protocol was in line with the rules determined by Dicle University, Professor Sabahattin Payzın Health Sciences, Research and Application Center, Animal Experiments Local Ethics Board. In addition, the animal experiment protocol was approved by the local ethics committee of Dicle University.

In the study, two microwave radiofrequency (RF) signal generators (Everest, Adapazari, Turkey) that emit 1800 and 2100 MHz GSM-like pulse signals were used for MWR exposure of rats [Alkis et al., 2019a; Dasdag et al., 2015]. Our 1800 and 2100 MHz signal generators create a GSM signal identical to that of a mobile phone, i.e. one pulse every 4.615 ms, giving a pulse repetition

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rate of 216.7 Hz and pulse duration of 0.577 ms. Dummy data are encoded using a variant of phase modulation called Gaussian Shift Keying (GMSK) with a symbol duration of approximately 4.6 ms. During the exposure, the rats were released in plexiglass cages and the generator was turned on. MWR was applied to the microwave application rats' group each day for 7 months and 2 h/day via the antennas of generators put in the middle of the lexiglass carousel (Fig. 1). The ventilation holes of the Plexiglass carousel allow air exchange between the exposure system and the environment. The applications were performed at the same time each day of the experiment, and the same protocol was used in the sham-control group rats aside from microwave application. The experiments were done in parallel in different rooms. The electric field (V/m) and electromagnetic power density (mW/cm^2) were determined by the measurement device EMR 300 (NARDA, Pfullingen, Germany). The mean±standard deviation power density and electric field values in plexiglass cages were measured respectively as 0.127 ± 0.04 mW/cm^2, 22.44 ± 4.02 V/m at 1800 MHz frequency and 0.038 ± 0.03 mW/cm^2, 11.52 ± 3.64 V/m at 2,100 MHz frequency. After the last application, all the rats that were taken to study were decapitated and the livers were excised rapidly. The liver tissue samples were transferred to the cooler at −80 °C and stored there until the day of analysis. Then, the DNA strand damage, MDA, 8-OHdG, and total oxidant-antioxidant analyses were done on the liver tissue. All tests, analyses, and assessments were carried out by individuals who were unaware of the groups so that the resulting research could be carried out blindly.

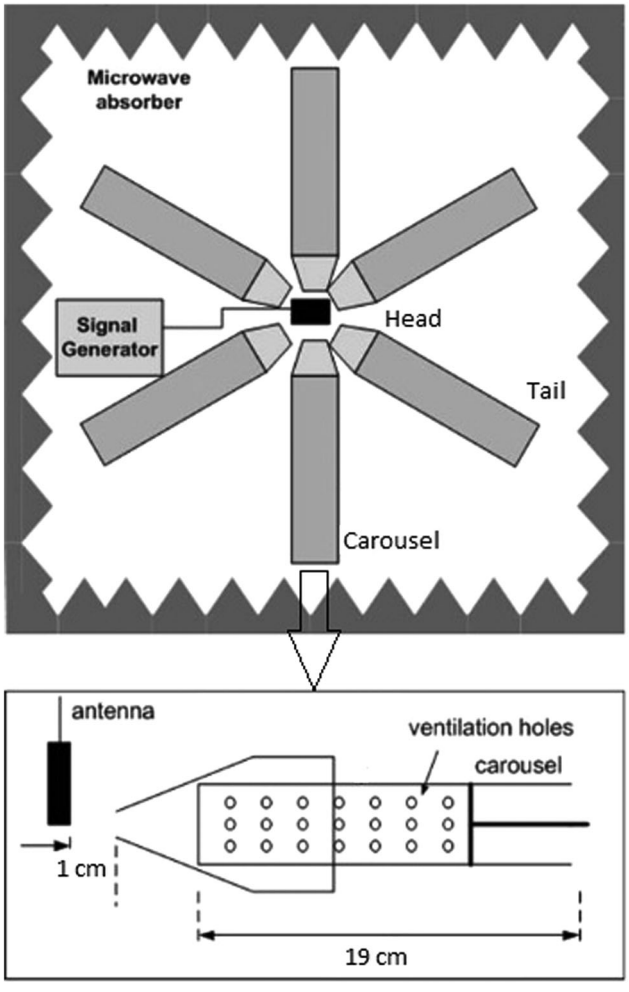


Fig. 1. Schematic drawing of the microwave radiation exposure system.

Specific Absorption Rate (SAR) Measurements

The average whole-body SAR values for 1800 and 2100 MHz are 0.62 and 0.20 W/kg, respectively (SAR value was determined as described by Alkis et al. [2019b]). The EMR 300 field probe was used to calculate the EMF values near and around the antenna. Although these measurements ensured the appropriate functioning of signal generators, they also provided the basis for the electromagnetic simulation of rats for SAR analysis, where the calculated electric field values were used as a reference in the simulations to simulate the average input power to the antenna. EMF simulations were performed by using CST Microwave Studio (Computer Simulation Technology [CST], Darmstadt, Germany).

In SAR simulations, the rat model plays a crucial part. The volumetric pixel (voxel) rat model that was formed used computerized tomography scans of a rat (commercially available). In the Voxel model, each tissue has its thermal and electrical properties measured at cell phone frequencies. Therefore, this voxel model is extremely specific and similar voxel sets for several human models also exist for industry-standard SAR calculations. The electric field and SAR distribution were simulated using CST with this rat voxel model. The simulated electric field values around the rats were consistent with the measured data using an electric field probe (NARDA, Pfullingen, Germany). SAR calculation was based on a 280 g rat, which is the average mass of a rat during the period of exposure.

Comet Assay Analysis

Lysis buffer (146.1 g $NaCl_2$, 37.2 g ethylenediaminetetraacetic acid [EDTA], 1.2 g trisma base, 1% triton-X, pH: 10), electrophoresis buffer (12 g NaOH [0.3 M], 0.372 g EDTA (triplex) [E2] (1 mM),

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pH: 13), low melting agar (0.6% low melting agar, phosphate-buffered saline [PBS]), and normal melting agar (1% agar, PBS) solutions were prepared for implementation in this study. The alkaline comet assay protocol was used for this analysis based on the technique of Singh et al. [1988]. Clean and unused slides were dipped into the normal melting agar and removed for drying. After the slides dried, the cell suspension was mixed with low melting agar (10 µl cell suspension + 90 µl agar) and placed on the slides, then closed with lamellae. The slides were then stored at +4 °C for 5 min. The lamellas on the slides were gently removed. All slides were kept in lysis buffer at 4 °C for 50 min. Following the lysis process, all slides were washed three times with PBS (pH: 7.4). We placed the slides that we washed before in the electrophoresis chamber and put 1 L of electrophoresis buffer into the chamber, then incubated for 40 min. At the end of the period, gels were executed at 25 V and 300 mA for 18 min. Following the electrophoresis process, the slides were washed two times with PBS, and once with distilled water. Ethidium bromide (2 mg/ml) was added to the slides and covered with lamella, then examined by a fluorescent microscope (Leica DM1000 LED; Leica Microsystems, Wetzlar, Germany). The microscopic examination process was implemented in the dark [Alkis et al., 2019a]. The scoring was assessed by using the Comet Assay IV software program (Perceptive Instruments, Suffolk, UK). The tail intensity (DNA% in the comet tail) and tail moment values were used to determine the damage to DNA. DNA strand breaks were analyzed by examining 100 DNA of cells in each sample.

Total Oxidant-Antioxidant Measurement and Calculation of Oxidative Stress Index

Total antioxidant status (TAS) is the total effect of all antioxidants, and total oxidant status (TOS) is the total effect of all plasma and body fluid oxidants. TAS and TOS measurements were performed with fully automated colorimetric methods of Erel [2004, 2005]. Liver tissue samples of the rats were homogenized by using PBS (pH: 7.4). In all tissue samples, the total protein content was determined using the Bradford method with a spectrophotometer (NanoDrop Technologies, Wilmington, DE). In tissue homogenates, TAS and TOS were measured in plaque readers at 660 and 530 nm wavelengths, respectively (Thermo Scientific Multiskan FC, Waltham, MA). The oxidative Stress Index was calculated using measured TAS and TOS values. The percent ratio of the TOS to the TAS gives the oxidative stress index (OSI), which is an indicator of the degree of oxidative stress [Erel, 2005].

Measurement of MDA

In the MDA measurement, the commercial MDA ELISA kit (Catalog no: CK-E10376; Eastbiopharm, Hangzhou, China) was used and the values were read by the enzyme-linked immunosorbent assay (ELISA) method on the plate reader (Thermo Scientific Multiskan FC). The samples and kit were brought to room temperature before starting work. A total of six pieces of standards were obtained by the serial dilution of the 64 nmol/ml stock standard that was taken from the kit. Fifty microliters of prepared standards were added to the microplate wells coated with antibody. Then 50 µl of Streptavidin-HRP (Biocompare, San Francisco, CA) was placed on the standards, which were pipetted into the microplate; 40 µl of serum and tissue homogenate samples were pipetted into each well. Ten microliters of MDA-antibody and 40 µl of Streptavidin-HRP (Biocompare) were placed on the samples, respectively. The microplate was closed and incubated at 37 °C for 60 min. After preparing the 30× of washing solution which existed in the kit, it was washed four times at 350 µl in the ELISA plate washer (Thermo Fisher Scientific, Waltham, MA). Each well was pipetted with 50 µl of Chromogen Solution A and B (Thermo Fisher Scientific), respectively. It was incubated at 37 °C for 10 min without filtering light. Each well was given 50 µl of Stop Solution (Thermo Fisher Scientific). The microplate was read at 450 nm absorbance in 10 min (Thermo Scientific Multiskan FC).

Measurement of 8-OHdG

8-OHdG is a biological marker of oxidative stress and carcinogenesis and one of the most important results of DNA oxidation. The 8-OHdG was measured by employing the 8-OHdG ELISA kit (Catalog no: CK-E11652; Eastbiopharm) and the values were read in the plate reader (Thermo Scientific Multiskan FC) by the ELISA technique. First, the samples and kit were brought to room temperature. Six pieces of standards were obtained by 128 ng/ml serial dilution of the stock standard taken from the kit. Fifty microliters of prepared standards were added to the microplate wells covered with an antibody. Then, 50 µl of Streptavidin-HRP was placed on the standards, which were pipetted to the microplate; 40 µl of serum and tissue homogenate samples were pipetted in each well. Ten microliters of 8-OHdG-antibody and 40 µl of Streptavidin-HRP were placed on the

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TABLE 1. Results of Tail Moment and Tail Intensity

	Group 1 (Sham-control)	Group 2 (1800 MHz)	Group 3 (2,100 MHz)	*P*
Tail moment (mean ± SD)	43.36 ± 7.6^{A}	50.34 ± 9.2^{A}	52.59 ± 10.04^{A}	0.2285
Tail intensity (Mean ± SD)	21.54 ± 2.99^{B}	31.40 ± 9.73^{A}	33.79 ± 8.83^{A}	0.0001

Different letters indicate statistical difference between the groups, while the same letters indicate no difference between groups. Tail intensity of exposed groups was found to be statistically significant according to sham-control group.

samples, respectively. The microplate was covered and incubated at 37°C for 60 min. Then, it was washed four times at 350 µl in the ELISA plate washer. Each well was pipetted with 50 µl of Chromogen Solution A and B, respectively. It was incubated for 10 min at 37 °C in the dark. Each well was given 50 µl of stop solution. The microplate was read in the plate reader (Thermo Scientific Multiskan FC) at 450 nm absorbance within 10 min. Showing the absorbance on the *x*-axis and the concentration on the *y*-axis, the line-line graphs were obtained by determining the absorbance of the standards, and results were expressed as ng/ml.

Statistical Analysis

SPSS statistical software program (version 25.0 software; SPSS, Chicago, IL) was applied in the analyses. Whether the data were compatible with normal distribution was determined with the Kolmogorov–Smirnov test, and it was found that they were normally distributed. The one-way variant analysis was used to determine if there was a difference between the groups. As a result of the variance analysis, Duncan multiple comparison tests were used to determine which groups were different in terms of variables. The results were presented as mean ± SD and a $P < 0.05$ was taken to be significant at a statistical level.

RESULTS

Results of Comet Assay

Results of the comet tail intensity and tail moment in the liver of rats are given in Table 1 as mean ± SD. There was a statistically significant increase in the tail intensity of exposed groups compared with the sham-control group ($P < 0.05$). However, it was determined that there was no significant difference in tail moment between the exposed- and sham-exposed groups ($P > 0.05$). In several studies, the tail intensity has been reported to provide more reliable results, and therefore it can be used for alkaline comet analyses [Kennedy et al., 2011]. The findings of comet assay parameters show that it is possible to identify the existence of DNA strand breaks at 1800 and 2100 MHz frequencies when the rats were exposed to 1800 and 2100 MHz MWR for 7 months. The example images of comet assay, which indicate DNA strand breaks in liver, are given in Figure 2.

Results of "TOS, TAS, OSI, MDA, and 8-OHdG"

The indicators of oxidative stress, lipid peroxidation, and oxidative DNA damage, "TOS, TAS, OSI, MDA, and 8-OHdG" analysis, are presented in Table 2 as mean ± SD ($n = 6$). As shown in Table 2,

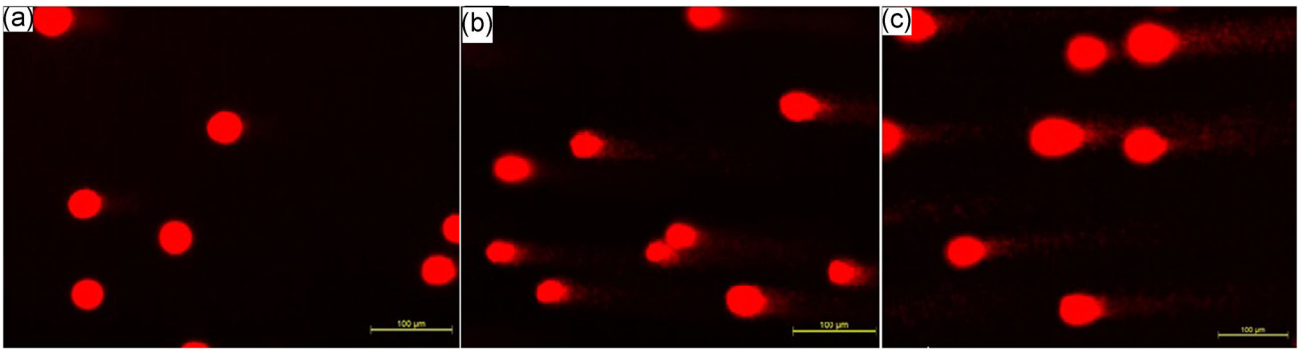


Fig. 2. Representative examples of comet images of liver cells; (**a**) sham-control, (**b**) 1800 MHz microwave radiation (MWR) exposure group, and (**c**) 2,100 MHz MWR exposure group. Comets indicate DNA damage in the liver of rats exposed to low-intensity MWR. Whole-cell heads without comets were considered to be undamaged.

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TABLE 2. Results of "TOS, TAS, OSI, 8-OHdG, and MDA"

	Group 1 (Sham)	Group 2 (1800 MHz)	Group 3 (2,100 MHz)	*P*
TOS (μmol/L)	7.95 ± 0.67^{D}	12.18 ± 0.81^{A}	10.66 ± 0.76^{B}	0.0001
TAS (μmol/L)	0.98 ± 0.27^{A}	0.74 ± 0.05^{B}	0.58 ± 0.11^{B}	0.0002
OSI (AU)	8.57 ± 2.28^{B}	16.52 ± 2.22^{A}	19.13 ± 4.10^{A}	0.0001
8-OHdG (ng/ml)	23.50 ± 18.46^{C}	46.87 ± 6.58^{A}	53.39 ± 12.61^{A}	0.0041
MDA (ng/ml)	32.83 ± 4.63^{C}	54.56 ± 9.44^{A}	43.72 ± 9.95^{B}	0.0001

Different letters indicate a statistical difference between the groups, while the same letters indicate no difference between groups. Statistically significant differences were observed in the parameters of exposure groups according to the sham-control group.
8-OHdG = 8-hydroxydeoxyguanosine; MDA = malondialdehyde; OSI = oxidative stress index; TAS = total antioxidant status; TOS = total oxidant status.

There was a statistically significant increase observed in "TOS, OSI, 8-OHdG, and MDA" values of exposure groups, compared with the sham-control group ($P < 0.05$). The TAS levels of both groups exposed to low-intensity MWR decreased considerably ($P < 0.05$). When the exposure groups were compared with each other, higher OSI and MDA values were observed in Group 3, while higher TOS values were detected in Group 2. There was no significant difference between exposure groups in terms of TAS and 8-OHdG. The significant increase of "MDA, 8-OHdG, TOS, and OSI" levels in MW-exposed rats shows that this radiation may function as genotoxic agent.

DISCUSSIONS

Humans are exposed to MWR with various frequencies and intensities for prolonged periods of time because of the widespread use of wireless devices in parallel with developing technology. The absorption of MWR in tissues depends on the dielectric properties and transmission of the tissue. As tissues such as the brain, kidney, lung, and liver have substantial quantities of water, they have higher conductivity than tissues holding less water such as bone and fat tissues [Ozgur et al., 2010]. Water constitutes a significant portion of cells and plays a determinant role in the cell metabolism, but the MWR is able to alter the physicochemical characteristics of the skin and subcutaneous water content [Ziskin, 2006]. Narinyan and Ayrapetyan [2017] reported that the non-thermal microwave (NTMW)-induced alterations in cell hydration can be a major messenger for the NTMW signal transduction from the cell aqua medium to intra-cellular metabolism. NTMW radiation causes R Na^{+}/Ca^{2+} exchange to activate, which leads to an increase in the intracellular Ca^{2+} [Narinyan and Ayrapetyan, 2017]. It has been revealed that EMF's effect on the physical and chemical characteristics of water solutions and formation of ROS changes depending on the gas composition of the medium, background radiation, temperature, Ca^{2+} concentration in water solution, and light [Ayrapetyan, 2015]. Mostly carrying and using cell phones in the pocket or on the belt close to the liver tissue, which is susceptible to radiation, has raised concerns on biological interactions between MWR and the liver [Ozguner et al., 2005]. Furthermore, in the literature, there are some conflicting findings on how DNA and oxidative damage is formed in different tissues exposed to MWR caused by cellphones, and this gap in the literature was the motivation for carrying out this study.

In this study, "Comet test, 8-OHdG, MDA, TAS, TOS, and OSI" analyses were performed to reveal whether exposure to low-intensity MWR emitted by mobile phones caused DNA and oxidative damage in the liver cells of rats. It was found that low-intensity MWR at 1800 MHz (SAR: 0.62 W/kg) and 2,100 MHz (SAR: 0.20 W/kg) frequencies increased the oxidative stress indicators "TOS, OSI, MDA, and 8-OHdG" levels in rats' liver cells and decreased TAS levels ($P < 0.05$). In addition, tail intensity, which is one of the DNA strand break indicators, increased significantly in the exposed groups compared with the sham-control group ($P < 0.05$).

Our results from different analysis tests confirm each other and are consistent with the findings of many previous studies [Akdag et al., 2016; Sieroń-Stołtny et al., 2017; Alkis et al., 2019a; Yang et al., 2020], suggesting that exposure to MWR can trigger DNA damage and oxidative stress. A number of experimental and epidemiological studies have revealed that excessive production of ROS occurs during high levels of MWR exposure and it causes health risks [Tomruk et al., 2009; Ozorak et al., 2013; Cetin et al., 2014; Alkis et al., 2019a,b]. Sieroń-Stołtny et al. [2017] reported that exposure to the radiofrequency EMF that is radiated by cell phones causes an intense pro-oxidant process and decreases antioxidant enzyme activity in the liver tissue of rats. Celik et al. [2016] stated that

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following the exposure of EMR at 2.45 GHz, the lipid peroxidation levels were raised in the liver and brain of rats, and that activity of glutathione peroxidase (GSH-Px), β-carotene, vitamin-E, and vitamin-A levels were reduced. Similarly, Ozgur et al. [2010] observed a significant increase in total nitric oxide (NO(x)) and MDA in the liver of the guinea pigs exposed to 1800 MHz GSM signal, and a significant decrease in the activities of GSH-Px, myeloperoxidase (MPO), and superoxide dismutase (SOD). In contrast to these findings, some researchers reported that no DNA or oxidative damage was observed in different rat tissues exposed to MWR emitted by cell phones [Zmyslony et al., 2004; Hirose et al., 2006; Dogan et al., 2012]. This inconsistency in the results of studies might be due to cell type as well as the different exposure parameters such as microwave frequency, exposure time, continuous or pulse wave, and SAR [Vijayalaxmi and Prihoda, 2012]. The damage mechanisms of tissues are thought to be relevant to ROS in biological systems impacted by MWR [Lai and Singh, 1996].

ROS are formed as the byproducts of cellular metabolism, especially in mitochondria. Some studies have asserted that EMFs cause an increase in Fenton reaction [Lai and Singh, 2004] and the activity of free radicals in cells [Lai and Singh, 1997]. Fenton reaction refers to the process of the conversion of hydrogen peroxide, which is produced by the mitochondria oxidative respiration, into highly cytotoxic hydroxyl free radicals [Phillips et al., 2009]. In case the cell's ROS production overwhelms its antioxidant capacity, damage may be observed in cellular macromolecules such as DNA, lipids, and proteins [Alkis et al., 2019a]. Oxidatively induced DNA damage, that is, DNA damage caused by ROS, could be repaired in the living cell by means of various repairing mechanisms [Ceylan et al., 2018]. Oxidative DNA changes that are not repaired before replication might lead to mutagenesis which is known to cause cancer [Friedberg, 2003; Dizdaroglu, 2015]. Genetic instability, which is induced by the mutations occurring in the genome, might have an effect on various types of enzymes in different pathways including DNA repair [Dizdaroglu, 2015]. There exists a balance between DNA repair and damage in healthy cells. But, in cancerous cells, this balance might be disrupted depending on DNA damage and the DNA-repairing capacity of cells that might be overwhelmed, resulting in high-frequency mutations [Akdag et al., 2016].

Lipid peroxidation is free radical-mediated oxidation of polyunsaturated fatty acids in biological membranes. Being a breakdown product of lipid peroxidation, MDA functions as a reliable marker for oxidative stress. The hydroxyl radical, which modifies the purine and pyrimidine bases in various ways, leads to the formation of numerous oxidized products. Being one of these oxidized products, 8-OHdG is highly abundant and mutagenic, which is why 8-OHdG serves as a direct indicator of oxidative DNA damage; and this approach has been usually used in determining oxidative DNA damage [Gurler et al., 2014]. There are many studies reporting that exposure to MWR causes an increase in 8-OHdG and MDA levels [Xu et al., 2010; Burlaka et al., 2013; Gurler et al., 2014; Guler et al., 2016; Sahin et al., 2016]. For instance, Guler et al. [2016] reported that 1800 MHz RFR caused lipid peroxidation and oxidative DNA damage (8-OHdG) in the brain and liver tissues of rabbits. However, there are also studies showing that MWR does not affect the 8-OHdG level [Khalil et al., 2012].

Our results show that 1800 and 2100 MHz high-frequency MWR exposure disrupts the oxidative balance in favor of free radicals and increases lipid peroxidation (MDA), and results in oxidative DNA damage (8-OHdG) in the liver tissue of rats. The findings of the research conducted by Khalil et al. [2012] are different from the findings of our study. We consider that this difference is due to the use of different SAR and biological materials. Oxidative DNA damage may result from an increase in free radical formation, impairment in DNA repair capability, and/or deterioration in productivity [Kundi et al., 2004]. In this manner, affecting the structural biomolecules in the cell, free radicals can be classified as one of the most important risk factors. We consider that GSM-like signals at 1800 and 2100 MHz can induce the modification of DNA base by way of increasing the activation of free radicals and suppressing antioxidants. It is well known that free radicals (hydroxyl) react with DNA components, and this reaction can cause serious damage to purine, pyrimidine components as well as the backbone deoxyribose [Phillips et al., 2009].

The endogenous and exogenous factors damage DNA unceasingly and, on the contrary, this damage is repaired by the repair enzymes. If an imbalance exists between damage and repair, DNA damage accumulates, which causes cancer, cell death, and aging. DNA lesions can be encountered in many different types. In this regard, DNA strand break is one of the most common lesions. Endogenous factors such as free radicals created by mitochondrial respiration and metabolism, and exogenous agents including non-ionized and ionized radiation, chemicals, and UV can cause the DNA strand break [Akdag et al., 2016].

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In the present study, low-intensity MWR radiation caused a significant increase in DNA strand break in liver tissues of rats, which is in line with the results of many studies [Lai and Singh, 1995, 1996, 1997; Trosic et al., 2011; Kesari et al., 2013; Smith-Roe et al., 2020]. Trosic et al. [2011] examined whether exposure to MWR in the frequency range of the cell phone (SAR: 0.6 W/kg; power density: 2.4 W/m^2; 915 MHz GSM) caused DNA damage in the kidney, liver, and brain tissues of rats by applying the Comet assay technique. They exposed the experimental animals to MWR 1 h each day for 2 weeks. Compared to the control group, tail length significantly increased in kidney and liver homogenates, but did not show a significant increase in brain cells in their examination. Kesari et al. [2013] reported that MWR radiated by a 3 G (2,100 MHz) cell phone could induce substantial DNA strand breaks in the brain.

Double- or single-strand breaks can be seen in DNA. Although the single-strand DNA break can be repaired by most cells, the double-strand DNA break can cause cell death or apoptosis, if not repaired in an appropriate way [Desai et al., 2009]. DNA damage is one of the key factors leading to cancer. The important increase in DNA strand breaks detected in liver tissue in this study shows that people should be careful in the prolonged usage of a cell phone, because DNA damage caused by MWR may trigger cancer development [Hardell et al., 2003].

When the exposure groups are compared with each other, Group 3 had higher OSI and MDA values, while higher TOS values were detected in Group 2. There was no significant difference between exposure groups in terms of "TAS, 8-OHdG, comet tail intensity, and tail moment." These findings suggest that the 2,100 MHz frequency is generally more dangerous than 1800 MHz, but radiation from either frequency can function as a genotoxic agent. The results of our present and previous research show that long-term exposure to low-intensity MWR can cause several health effects.

CONCLUSION

As a result, the outcomes of our study demonstrated that long-term exposure to 1800 and 2100 MHz, low-intensity MWR emitted by cell phones can change oxidant-antioxidant parameters and lead to DNA strand breaks, lipid peroxidation, and oxidative DNA damage in the liver of rats. Although MW energy is not directly sufficient to break DNA strand breaks, genotoxic effects might be induced by indirect mechanisms such as DNA repair functions or free radicals' generation. Knowledge of the potential health impact of MWR is still insufficient and inconclusive. To reach a definitive conclusion, further research is needed.

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REFERENCES

Akdag M, Dasdag S, Canturk F, Akdag MZ. 2018. Exposure to non-ionizing electromagnetic fields emitted from cell phones induced DNA damage in human ear canal hair follicle cells. Electromagn Biol Med 37:66–75.

Akdag MZ, Dasdag S, Canturk F, Karabulut D, Caner Y, Adalier N. 2016. Does prolonged radiofrequency radiation emitted from Wi-Fi devices induce DNA damage in various tissues of rats? J Chem Neuroanat 75:116–122.

Alkis ME, Bilgin HM, Akpolat V, Dasdag S, Yegin K, Yavas MC, Akdag MZ. 2019a. Effect of 900-, 1800-, and 2100-MHz radiofrequency radiation on DNA and oxidative stress in brain. Electromagn Biol Med 38:32–47.

Alkis ME, Akdag MZ, Dasdag S, Yegin K, Akpolat V. 2019b. Single-strand DNA breaks and oxidative changes in rat testes exposed to radiofrequency radiation emitted from cellular phones. Biotechnol Biotechnol Equip 33:1733–1740.

Ayrapetyan S, De J. 2014. Cell hydration as a biomarker for estimation of biological effects of nonionizing radiation on cells and organisms. Sci World J 2014:1–8.

Ayrapetyan G, Papanyan A, Hayrapetyan H, Ayrapetyan S. 2005. Metabolic pathway of magnetized fluid-induced relaxation effects on heart muscle. Bioelectromagnetics 26:624–630.

Ayrapetyan S. 2015. The role of cell hydration in realization of biological effects of non-ionizing radiation (NIR). Electromagnet Biol Med 34:197–210.

Ayrapetyan S. 2017. The intracellular signaling system controlling cell hydration as a biomarker for EMF dosimetry. In: Markov M, editor, *Dosimetry in Bioelectromagnetics*. Boca Raton, FL: CRC Press. pp 339–366.

Bhargava S, Motwani MB, Patni VM. 2012. Effect of handheld mobile phone use on parotid gland salivary flowrate and volume. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 114:200–206.

Burlaka A, Tsybulin O, Sidorik E, Lukin S, Polishuk V, Tsehmistrenko S, Yakymenko I. 2013. Overproduction of free radical species in embryonal cells exposed to low intensity radiofrequency radiation. Exp Oncol 35:219–225.

Celik O, Kâhya MC, Nazıroğlu M. 2016. Oxidative stress of brain and liver is increased by Wi-Fi (2.45 GHz) exposure of rats during pregnancy and the development of newborns. J Chem Neuroanat 75:134–139.

Cetin H, Nazıroğlu M, Celik O, Yüksel M, Pastacı N, Ozkaya MO. 2014. Liver antioxidant stores protect the brain from electromagnetic radiation (900 and 1800 MHz)-induced oxidative stress in rats during pregnancy and the development of offspring. J Matern Fetal Neonatal Med 27:1915–1921.

Ceylan D, Tuna G, Kirkali G, Tunca Z, Can G, Arat HE, Kant M, Dizdaroglu M, Özerdem A. 2018. Oxidatively-induced

1521186x, 2021, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/bem.22315 by University Of British Columbia, Wiley Online Library on [10/10/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

DNA damage and base excision repair in euthymic patients with bipolar disorder. DNA Repair 65:64–72.
Chaplin MF. 2006. Information Exchange within Intracellular Water. In: Pollack GH, Cameron IL, Wheatley DN, editors. Water and the Cell, First Edition, Dordrecht, Netherlands: Springer Press., First Ed, 113–124.
Chauhan P, Verma HN, Sisodia R, Kesari KK. 2017. Microwave radiation (2.45 GHz)-induced oxidative stress: Whole-body exposure effect on histopathology of Wistar rats. Electromagn Biol Med 36:20–30.
Dasdag S, Akdag MZ. 2016. The link between radio-frequencies emitted from wireless technologies and oxidative stress. J Chem Neuroanat 75:85–93.
Dasdag S, Akdag MZ, Erdal ME, Erdal N, Ay OI, Ay ME, Yilmaz SG, Tasdelen B, Yegin K. 2015. Long term and excessive use of 900 MHz radiofrequency radiation alter microRNA expression in brain. Int J Radiat Biol 91:306–311.
Desai NR, Kesari KK, Agarwal A. 2009. Pathophysiology of cell phone radiation: Oxidative stress and carcinogenesis with focus on male reproductive system. Reprod Biol Endocrinol 7:114.
Dizdaroglu M. 2015. Oxidatively induced DNA damage and its repair in cancer. Mutat Res 763:212–245.
Dogan M, Turtay MG, Oguzturk H, Samdanci E, Turkoz Y, Tasdemir S, Alkan A, Bakir S. 2012. Effects of electromagnetic radiation produced by 3G mobile phones on rat brains: Magnetic resonance spectroscopy, biochemical, and histopathological evaluation. Hum Exp Toxicol 31: 557–564.
Eid FA, El-Gendy AM, Zahkouk SA, El-Tahway NA, El-Shamy SA. 2015. Examination of studies on the subject shows that the results of EMF applied at different frequencies and for different durations vary in different tissues. Egypt J Hosp Med 58:74–93.
Erel O. 2004. A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem 37:112–119.
Erel O. 2005. A new automatedri colometric method for measuring total oxidant status. Clin Biochem 38:1103–1111.
Esmekaya MA, Sırav B, Ozer C, Seyhan N. 2011. Effects of radiofrequency radiation on brain tissue oxidant and antioxidant levels in rats. Gazi Med J 22:100–104.
Friedberg EC. 2003. DNA damage and repair. Nature 421: 436–440.
Gapeyev AB, Mikhailik EN, Chemeris NK. 2009. Features of anti-inflammatory effects of modulated extremely high-frequency electromagnetic radiation. Bioelectromagnetics 30:454–461.
Guler G, Tomruk A, Ozgur E, Seyhan N. 2010. The effect of radiofrequency radiation on DNA and lipid damage in non-pregnant and pregnant rabbits and their newborns. Gen Physiol Biophys 29:59–60.
Guler G, Ozgur E, Keles H, Tomruk A, Vural SA, Seyhan N. 2016. The neurodegenerative changes and apoptosis induced by intrauterine and extrauterine exposure of radiofrequency radiation. J Chem Neuroanat 75:128–133.
Gurler HS, Bilgici B, Akar AK, Tomak L, Bedir A. 2014. Increased DNA oxidation (8-OHdG) and protein oxidation (AOPP) by low level electromagnetic field (2.45 GHz) in rat brain and protective effect of garlic. Int J Radiat Biol 90:892–896.
Halliwell B, Gutteridge JMC. 2015. Free Radicals in Biology and Medicine: Oxidative Stress and Redox Regulation: Adaptation, Damage, Repair, Senescence, and Death, First Edition, New York, NY: Oxford University Press. pp 905–961.
Hardell L, Mild KH, Johansson B. 2003. Cellular and cordless telephones and basal cell carcinoma. Arch Environ Occup Health 58:380–382.
Hirose H, Sakuma N, Kaji N, Suhara T, Sekijima M, Nojima T, Miyakoshi J. 2006. Phosphorylation and gene expression of p53 are not affected in human cells exposed to 2.1425 GHz band CW or W-CDMA modulated radiation allocated to mobile radio base stations. Bioelectromagnetics 27: 494–504.
Kennedy EK, McNamee JP, Prud'homme Lalonde L, Jones T, Wilkinson D. 2011. Acellular comet assay: A tool for assessing variables influencing the alkaline comet assay. Radiat Prot Dosim 148:155–161.
Kesari KK, Meena R, Nirala J, Kumar J, Verma HN. 2013. Effect of 3G cell phone exposure with computer controlled 2-D stepper motor on non-thermal activation of the hsp27/p38MAPK stress pathway in rat brain. Cell Biochem Biophys 68:347–358.
Khalil AM, Gagaa MH, Alshamali AM. 2012. 8-Oxo-7, 8-dihydro-2′-deoxyguanosine as a biomarker of DNA damage by mobile phone radiation. Hum Exp Toxicol 31:734–740.
Kundi M, Mild KH, Hardell L, Mattsson MO. 2004. Mobile telephones and cancer a review of epidemiological evidence. J Toxicol Environ Health Part B 7:351–384.
Lai H, Singh NP. 1995. Acute low-intensity microwave exposure increases DNA single-strand breaks in rat brain cells. Bioelectromagnetics 16:207–210.
Lai H, Singh NP. 1996. Single-and double-strand DNA breaks in rat brain cells after acute exposure to radiofrequency electromagnetic radiation. Int J Radiat Biol 69:513–521.
Lai H, Singh NP. 1997. Melatonin and a spin-trap compound block radiofrequency electromagnetic radiation-induced DNA strand breaks in rat brain cells. Bioelectromagnetics 18:446–454.
Lai H, Singh NP. 2004. Magnetic-field-induced DNA strand breaks in brain cells of the rat. Environ Health Perspect 112:687–694.
Meo SA, Arif M, Rashied S, Husain S, Khan MM, Al Masri AA, Vohra MS, Usmani AM, Husain A, Al-Drees AM. 2010. Morphological changes induced by mobile phone radiation in liver and pancreas in Wistar albino rats. Eur J Anat 14:105–109.
Narinyan L, Ayrapetyan S. 2017. Cyclic AMP-dependent signaling system is a primary metabolic target for non-thermal effect of microwaves on heart muscle hydration. Electromagn Biol Med 36:182–191.
Ozguner F, Oktem F, Armagan A, Yilmaz R, Koyu A, Demirel R, Vural H, Uz E. 2005. Comparative analysis of the protective effects of melatonin and caffeic acid phenethyl ester (CAPE) on mobile phone-induced renal impairment in rat. Mol Cell Biochem 276:31–37.
Ozgur E, Güler G, Seyhan N. 2010. Mobile phone radiation-induced free radical damage in the liver is inhibited by the antioxidants n-acetyl cysteine and epigallocatechin-gallate. Int J Radiat Biol 86:935–945.
Ozorak A, Nazıroğlu M, Celik O, Yüksel M, Ozcelik D, Ozkaya MO, Cetin H, Kahya MC, Kose SA. 2013. Wi-Fi (2.45 GHz)- and Mobile phone (900 and 1800 mhz)-induced risks on oxidative stress and elements in kidney and testis of rats during pregnancy and the development of offspring. Biol Trace Elem Res 156:221–229.

1521186x, 2021, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/bem.22315 by University Of British Columbia, Wiley Online Library on [10/10/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Phillips JL, Singh NP, Lai H. 2009. Electromagnetic fields and DNA damage. Pathophysiology 16:79–88.

Sahin D, Ozgur E, Guler G, Tomruk A, Tomruk A, Unlu I, Sepici-Dinçel A, Seyhan N. 2016. The 2100 MHz radiofrequency radiation of a 3G-mobile phone and the DNA oxidative damage in brain. J Chem Neuroanat 75:94–98.

Sieroń-Stołtny K, Pasek J, Cieślar G, Sieroń A. 2017. Influence of electromagnetic fields on prooxidant/antioxidant balance in rat liver. Pol J Environ Stud 26:279–285.

Singh NP, McCoy MT, Tice RR, Schneider EL. 1988. A simple technique for quantization of low levels of DNA damage in individual cells. Exp Cell Res 175:184–191.

Smith-Roe SL, Wyde ME, Stout MD, Winters JW, Hobbs CA, Shepard KG, Green AS, Kissling GE, Shockley KR, Tice RR, Bucher JR, Witt KL. 2020. Evaluation of the genotoxicity of cell phone radiofrequency radiation in male and female rats and mice following subchronic exposure. Environ Mol Mutagen 61:276–290.

Tomruk A, Guler G, Dincel AS. 2009. The influence of 1800 MHz GSM-like signals on hepatic oxidative DNA and lipid damage in nonpregnant, pregnant, and newly born rabbits. Cell Biochem Biophys 56:39–47.

Topal Z, Hanci H, Mercantepe T, Erol HS, Keles ON, Serkan H. 2015. The effects of prenatal long-duration exposure to 900-MHz electromagnetic field on the 21-day-old newborn male rat liver. Turk J Med Sci 45:291–297.

Trosic I, Pavicic I, Milkovic-Kraus S, Mladinić M, Zeljezić D. 2011. Effect of electromagnetic radiofrequency radiation on the rats' brain, liver and kidney cells measured by comet assay. Coll Antropol 35:1259–1264.

Tsikas D. 2017. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. Anal Biochem 524:13–30.

Vijayalaxmi, Prihoda TJ. 2012. Genetic damage in human cells exposed to non-ionizing radiofrequency fields: A meta-analysis of the data from 88 publications (1990–2011). Mutat Res-Gen Tox En 749:1–16.

Xu S, Zhou Z, Zhang L, Yu Z, Zhang W, Wang Y, Wang X, Li M, Chen Y, Chen C, He M, Zhang G, Zhong M. 2010. Exposure to 1800 MHz radiofrequency radiation induces oxidative damage to mitochondrial DNA in primary cultured neurons. Brain Res 1311:189–196.

Yakymenko I, Tsybulin O, Sidorik E, Henshel D, Kyrylenko O, Kyrylenko S. 2015. Oxidative mechanisms of biological activity of low-intensity radiofrequency radiation. Electromagn Biol Med 35:186–202.

Yang H, Zhang Y, Wang Z, Zhong S, Hu G, Zuo W. 2020. The effects of mobile phone radiofrequency radiation on cochlear stria marginal cells in Sprague–Dawley rats. Bioelectromagnetics 41:219–229.

Yokus B, Cakir DU, Akdag MZ, Sert C, Mete N. 2005. Oxidative DNA damage in rats exposed to extremely low frequency electro magnetic fields. Free Radic Res 39:317–323.

Ziskin MC. 2006. Physiological mechanisms underlying millimeter wave therapy. In: Ayrapetyan SN, Markov MS, editors, *Bioelectromagnetics Current Concepts*. Dordrecht, the Netherlands: Springer. pp 241–251.

Zmyslony M, Politanski P, Rajkowska E, Szymczak W, Jajte J. 2004. Acute exposure to 930 MHz CW electromagnetic radiation in vitro affects reactive oxygen species level in rat lymphocytes treated by iron ions. Bioelectromagnetics 25:324–328.

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